

IMMEDIATE COMMUNICATION

Copy number variation in schizophrenia in Sweden

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Schizophrenia (SCZ) is a highly heritable neuropsychiatric disorder of complex genetic etiology. Previous genome-wide surveys have revealed a greater burden of large, rare copy number variations (CNVs) in SCZ cases and identified multiple rare recurrent CNVs that increase risk of SCZ although with incomplete penetrance and pleiotropic effects. Identification of additional recurrent CNVs and biological pathways enriched for SCZ CNVs requires greater sample sizes. We conducted a genome-wide survey for CNVs associated with SCZ using a Swedish national sample (4719 cases and 5917 controls). High-confidence CNV calls were generated using genotyping array intensity data, and their effect on risk of SCZ was measured. Our data confirm increased burden of large, rare CNVs in SCZ cases as well as significant associations for recurrent 16p11.2 duplications, 22q11.2 deletions and 3q29 deletions. We report a novel association for 17q12 duplications (odds ratio = 4.16, $P = 0.018$), previously associated with autism and mental retardation but not SCZ. Intriguingly, gene set association analyses implicate biological pathways previously associated with SCZ through common variation and exome sequencing (calcium channel signaling and binding partners of the fragile X mental retardation protein). We found significantly increased burden of the largest CNVs (>500 kb) in genes present in the postsynaptic density, in genomic regions implicated via SCZ genome-wide association studies and in gene products localized to mitochondria and cytoplasm. Our findings suggest that multiple lines of genomic inquiry—genome-wide screens for CNVs, common variation and exonic variation—are converging on similar sets of pathways and/or genes.

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INTRODUCTION

Schizophrenia (SCZ) is an often devastating psychiatric disorder with substantial morbidity, mortality and personal and societal costs.^{1–3} An important genetic component is indicated by a sibling recurrence risk of 8.6, high heritability estimates (0.64 in a national family study, 0.81 in a meta-analysis of twin studies and 0.32 estimated directly from common single-nucleotide polymorphisms (SNPs)) and previous genomic findings.^{4–7}

Recent studies into the genetic architecture of this disease have identified both common and rare variation.^{7–13} Genome-wide association studies (GWAS) have implicated 22 genome-wide significant loci plus biological pathways, including genes regulated by miR-137, neuronal calcium channel signaling and binding partners of fragile X mental retardation protein (FMRP). Exome sequencing of 2536 SCZ cases and 2543 controls implicated gene sets enriched for rare exonic variations, including genes involved in calcium channel signaling, FMRP interactors and the neuronal activity-regulated cytoskeleton-associated complex of the post-synaptic density (PSD).¹⁴ Genomic evaluation of copy number variation (CNV) has established a role for large rare CNVs (>100 kb, <1%) in risk for SCZ. Multiple studies have reported a greater burden of rare CNVs in SCZ cases versus controls.^{15–17} Eight rare CNVs of strong effect (odds ratio (OR) 4–20) increase risk for SCZ (for example, 22q11del and 16p11dup) and are often

recurrent mutations in genomic hotspots with incomplete penetrance and pleiotropy.^{10,11,13} Enrichment analyses of genes intersected by rare CNVs implicated functional categories related to synaptic activity and neurodevelopment and components of the PSD (particularly the N-methyl-D-aspartate receptor and activity-regulated cytoskeleton-associated complexes).^{12,15,18}

The cumulative results from genomic studies of common and rare variation strongly suggest that SCZ is notably polygenic (that is, many genes of differing effect sizes confer the risk for SCZ). Besides the eight CNVs of strongest effects,¹³ it remains unclear whether additional CNVs contribute to the risk for SCZ. Larger samples are required to yield new insights and identify novel loci of lower frequencies or modest effects.

Of particular interest, several gene sets (for example, calcium channel signaling and FMRP interactors) have convergent genomic results from GWAS and exome sequencing. This convergence from different methodologies minimizes risk of bias and suggests that different types of alleles can perturb the same biological pathways that contribute to the etiology of SCZ. However, it remains unclear whether CNVs similarly have a role in these pathways of high risk. Previous enrichment analyses of genes intersected by rare CNVs did not explicitly examine the relationship between rare CNVs and common SNPs.

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The purpose of this study was to identify CNV alleles, genes or gene sets that confer risk for SCZ in a well-powered sample and to examine the overlap and relative impact of common SNPs and rare CNVs. We conducted a genome-wide CNV survey in a Swedish sample (4719 cases with SCZ and 5917 controls)⁷. This sample is well suited for this study given its national sampling frame and relative homogeneity. Although the primary data source were genome-wide SNP arrays, all samples were also genotyped with Illumina exome arrays enabling CNV validation with an independent technology.¹⁹

MATERIALS AND METHODS

Additional information is in the Supplementary Methods. To advance knowledge of SCZ, CNV data on subsamples were included in previous reports.^{20,21} We present here the primary analyses of this project and note that CNV data for 57% of the sample have never been reported before.

Subjects

Subject ascertainment, diagnosis and validation are described elsewhere⁷ and summarized in the Supplementary Methods. Briefly, all procedures were approved by ethical committees in Sweden and the US, and all subjects provided written informed consent. Cases with SCZ were identified using the Swedish Hospital Discharge Register,^{22,23} which captures all public and private inpatient hospitalizations.^{24–27} Case inclusion criteria: ≥ 2 hospitalizations with a discharge diagnosis of SCZ, both parents born in Scandinavia, and age ≥ 18 years. Case exclusion criteria: hospital register diagnosis of any medical or psychiatric disorder mitigating a confident diagnosis of SCZ. The validity of this case definition of SCZ is strongly supported as detailed in the Supplementary Note of reference.⁷ Controls were selected at random from Swedish population registers, with the goal of obtaining an appropriate control group and avoiding 'super-normal' controls that can cause substantial bias in psychiatric research.^{28,29} Control inclusion criteria: never hospitalized for SCZ or bipolar disorder, both parents born in Scandinavia, and age ≥ 18 years. Participation rates were lower for cases than for controls (53.3 versus 58.3%) but similar to participation rates in epidemiology (41% for cross-sectional and 56% for case-control studies)^{30,31} and a large Norwegian longitudinal study (42%).

Genotyping and quality control (QC)

DNA was extracted from peripheral venous blood for all subjects. Genotyping was done in six batches (Sw1–6) at the Broad Institute using Affymetrix 5.0 (Affymetrix, Inc., Santa Clara, CA, USA; 3.9%, Sw1), Affymetrix 6.0 (38.6%, Sw2–4) and Illumina OmniExpress (57.4%, Sw5–6). Genotypes were called using Birdsuite (Affymetrix) or BeadStudio (Illumina, Inc., San Diego, CA, USA). QC filters excluded SNPs for missingness ≥ 0.05 or minor allele frequency < 0.01 and subjects for missingness ≥ 0.02 , autosomal heterozygosity deviation and one of any pair of subjects with high relatedness ($\hat{r} > 0.2$). A total of 11 244 subjects (5001 cases with SCZ and 6243 controls) remained and were used for subsequent CNV calling and QC. All genomic locations are given in NCBI build 37/UCSC hg19 coordinates.

CNV calling and QC

We used Birdseye to detect CNVs.³² Birdseye applies a hidden Markov model to normalized probe intensities using model priors tuned to each GWAS array. Basic QC included removal of low-confidence CNVs with confidence scores < 10 , spanning < 10 probes or < 10 kb in length³² followed by removal of CNVs with $> 50\%$ reciprocal overlap with large genomic gaps (for example, centromeres) or regions subject to rearrangement in white blood cells. We annealed adjoining CNVs that appeared to be artificially split by Birdseye by recursively joining CNVs if the called region is $\geq 80\%$ of the entire region to be joined. The largest CNVs (≥ 5 Mb) and chrX CNVs were visually inspected and those of low confidence were removed. We excluded subjects whose genotyping arrays had excessive noise (probe intensity variance or genomic 'waviness' exceeding platform-specific thresholds, Supplementary Table S3, Supplementary Figure S1) or excessive CNV calls scattered across many chromosomes (≥ 40 segments or total length ≥ 6 Mb). These procedures resulted in a final sample size of 10 636 subjects (4719 cases and 5917 controls, Supplementary Table S4).

Because Birdseye was optimized to identify rare CNVs,³² we imposed a 0.01 frequency threshold by removing CNVs with $> 50\%$ of its length spanning a region with > 107 CNVs. Our main analyses were conducted using CNVs ≥ 100 kb and spanning ≥ 15 probes, which gave an estimated false-positive rate of 3.3% based on NanoString validation of 212 CNVs detected from Affymetrix 6.0 SNP arrays (unpublished data).

CNV validation

The same DNA samples from all cases and controls were genotyped on Illumina exome arrays. We developed CNV calling procedures for these data (essentially, an exon-focused set of 250K probes) and have shown that the exome array has high sensitivity and specificity to identify CNVs ≥ 400 kb.¹⁹ Therefore, we used these additional data for large-scale validation. A CNV (≥ 400 kb) is considered validated if it is overlapped by an exome array CNV in the same sample by 50% of its length. Supplementary Table S5 displays the validation results stratified by array type and for deletions and duplications separately and combined. The validation rates were 89, 83 and 92% for Affymetrix 5.0, Affymetrix 6.0 and Illumina Omni Express arrays, respectively. For all nominally associated CNVs that had sufficient probe coverage from the exome array, we manually inspected CNV calls and probe intensity plots in the same samples.

Statistical analysis

All analyses were conducted using PLINK³³ and R.³⁴ As confounders can create spurious associations, we tested a series of metrics (Supplementary Table S7) for their impact on genome-wide CNV burden using multiple logistic regression with genotyping batch, subject ancestry, age and sex included as covariates. As anticipated, genotyping batch (Sw1–6) could potentially influence association testing and was controlled for in all analyses. For each association test, 100 000 permutations were performed to evaluate statistical significance with the permutation procedure swapping case-control status within genotyping batches to control for batch effect.²⁰ As shown in Supplementary Table S4, each genotyping batch used one specific type of arrays. Thus controlling for genotyping batch effect simultaneously controlled for the difference in genotyping method as permutation swapped case-control status within a specific array type.

Genome-wide burden analysis. We conducted burden analyses across a range of CNV frequencies, sizes and types. The burden of rare CNVs was measured as the number of CNVs, the genomic length impacted by CNVs and the number of genes impacted by CNVs ('gene count') using one-sided statistical tests (assuming increased CNV burden in cases). ORs measure the increase in the likelihood of having disease per unit increase in CNV burden and were computed in R using logistic regression with batch as a covariate.

Known loci. Large CNVs with previously reported associations with SCZ, other psychiatric disorders or developmental delay (Table 2)^{10,11,13,35,36} were examined using regional association testing in PLINK for deletions and duplications separately. For single genes, (that is, *NRXN1*, *VIPR2*), we considered all > 100 kb CNV events that disrupted the gene (≥ 1 bp overlap). For all other regions, we considered all > 100 kb CNV events that had $> 50\%$ reciprocal overlap with a region (PLINK `-cnv-union-overlap 0.5`). ORs and confidence intervals (CIs) were computed in R. When zero events occurred, ORs were estimated by applying the standard continuity correction (that is, adding 0.5 to each cell of the 2×2 table).³⁷

Single-site and gene-based association. We excluded known CNV loci in an attempt to identify novel deletions or duplications. The single-site analysis compared the number of CNV events between cases and controls per marker, where PLINK defines markers as to the start and stop sites of all CNV segments. For gene-based association, we used Ensembl gene models (20 007 protein coding genes)³⁸ and, for each gene, we determined the counts of CNVs disrupting the gene (≥ 1 bp overlap) in cases versus controls. To select loci for validation and replication, we extracted nominally associated loci ($P < 0.01$) and excluded spurious CNVs by manually inspecting probe intensities and overlap with segmental duplications.

Statistical power. Power analyses to detect single CNV loci were conducted using the R/gap package (see URLs). Assuming a dominant model, lifetime risk of SCZ of 0.7%³⁹ and $\alpha = 0.05$, we computed the minimal detectable genotypic risk ratio to achieve 20, 25, 80, 90 or 95% power over

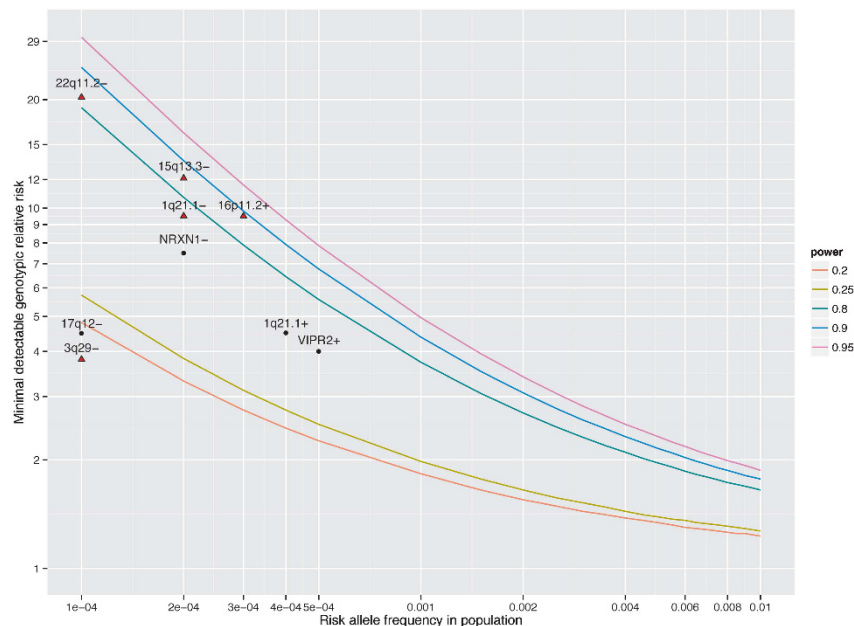


Figure 1. Power to detect novel and known risk loci. The three solid lines depict the minimal detectable genotypic relative risk (y axis) as a function of the risk allele frequency in the population (x axis) to achieve 20, 25, 80, 90 and 95% power. Assumptions were 4719 cases/5917 controls, a dominant model, lifetime risk of schizophrenia (SCZ) of 0.7% and $\alpha = 0.05$. An additive model produced nearly identical results. Nine known copy number variations associated with SCZ are shown as red diamonds ($P < 0.05$ in this study) or black dots (not detected). For each risk locus, the estimated odds ratio and allele frequency in controls were obtained from the literature. If not found in controls, allele frequencies were set to 0.0001. The four most replicated loci (22q11.2-, 15q13.3-, 1q21.1 and 16p11.2+) can be detected given our sample size. Loci 17q12-, 3q29-, 1q21.1+ and *VIPR2*+ likely have imprecise estimates of genotypic relative risk or frequency as evidenced in large discrepancies between various reports.

a range of frequency of risk alleles in the population (Figure 1). An additive model produced nearly identical results.

Gene set analyses. We evaluated the collective effects of rare CNVs in predefined sets of genes using the 'cnv-enrichment-test' in PLINK relative to all genic-CNVs (that is, CNVs overlapping any gene by ≥ 1 bp). This method explicitly compares the rate of CNVs impacting a specific gene set in cases versus controls while controlling for sources of bias.⁴⁰ Restricting to only genic CNVs ensures specificity of the enrichment to the set of interests.⁴⁰ Specifically, PLINK fits a logistic regression model: $\log \left[\frac{P_{i,case}}{1 - P_{i,case}} \right] = \theta + \beta_0 \cdot c_i + \beta_1 \cdot s_i + \gamma \cdot g_i$, where P_i is the probability that individual i is affected, c_i is the number of genic-CNVs that an individual i has, s_i is the mean size of those events measured in kb, g_i is the count of genes within a given gene set affected by genic-CNVs and other terms are logistic regression coefficients. The 'cnv-enrichment-test' tests if γ , the coefficient associated with gene counts, is significantly different from 0 and evaluates its statistical significance via 100 000 permutations swapping case-control status within genotyping batches to control for batch effect.²⁰ To estimate ORs for the increased risk of SCZ per affected gene, we fit the above logistic regression model with genotyping batch included as an additional covariate and computed e^γ , where γ is the estimated coefficient associated with gene counts after correcting for background difference in rate and size of genic-CNVs and genotyping batch effect.

To discover novel gene sets associated with SCZ, we used established pathways (Kyoto Encyclopedia of Genes and Genomes (KEGG), Gene Ontology (GO))^{41–45} and TargetScan (v6.2, predicted 3' untranslated region targets of micro-RNAs).⁴⁶ To assess gene sets previously implicated in SCZ and other psychiatric disorders, we analyzed neuronal calcium signaling genes, genes making RNAs that bind to FMRP (the product of *FMR1*)^{7,14,47} genes spanned by a *de novo* CNV in Kirov et al.,¹² genes making proteins found in the neuronal PSD,¹² expert-curated lists of synaptic genes,⁴⁸ the 'genes2cognition' database,⁴⁹ genes implicated in autism⁵⁰ or mental retardation,^{45,51–53} genes whose knock out in mouse

yields a neurological or behavioral phenotype^{54,55} and human nuclear-encoded mitochondrial genes in MitoCarta.⁵⁶ All gene sets were established *a priori* and independently of this work. Kirov et al.¹² reported that eight *de novo* CNVs overlapped four known SCZ-associated CNVs (3q29, 15q11.2, 15q13.3 and 16p11.2).

To correct for multiple comparisons in the discovery analysis (that is, KEGG, GO, TargetScan), we used the false discovery rate control as implemented in R/q-value.⁵⁷ Given the discovery nature of the analysis, false discovery rate is less conservative than Bonferroni approach and has greater power to find truly significant results while effectively reducing false positives. To correct for multiple comparisons in the analysis of gene sets previously implicated in SCZ, we applied the Holm-Bonferroni⁵⁸ method and acknowledge the conservativeness of this approach. The Holm-Bonferroni adjusted P -values (adj. P) were computed in R considering all 126 association tests performed to establish statistical significance. For each significant enrichment identified, we examined specific genes affected by CNVs driving the enrichment and their potential overlap with known CNVs/genes associated with SCZ and other psychiatric disorders.

Rare CNVs and significant GWAS loci. We evaluated whether CNVs in SCZ cases were enriched for smaller GWAS P -values. The GWAS P -values were obtained from a meta-analysis of the Swedish samples (5001 cases with SCZ and 6243 controls) and independent Psychiatric Genomic Consortium (PGC) samples (8832 cases with SCZ and 12 067 controls).⁷ There were 2691 genes with $P < 10^{-3}$. We applied a logistic regression model⁴⁰ for the likelihood of SCZ as a function of having a CNV overlapping any of these genes while accounting for background differences in the rate and size of genic CNVs. The extended major histocompatibility complex region (chr6:25–34 mb) was excluded, and the 'cnv-enrichment-test' in PLINK relative to all genic CNVs was used with permutation to control for batch effects.

Rare CNV and risk profile score (RPS) burden. We evaluated the relative impact of rare CNV burden and common variant allelic burden (that is, RPSs)^{7,20,59} on the risk of SCZ. RPSs were used in the 2011 PGC GWAS

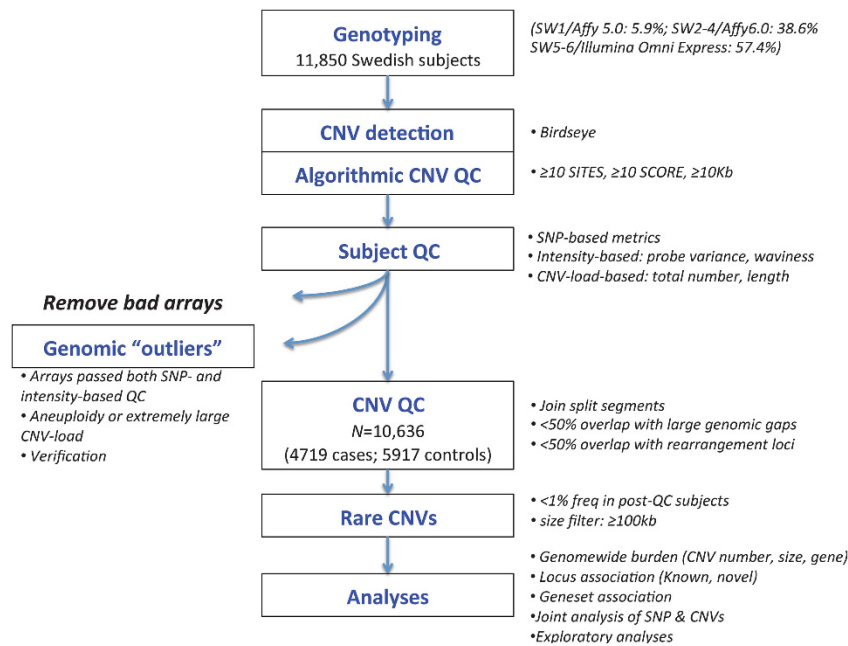


Figure 2. Experimental workflow and copy number variation (CNV) data sets. QC, quality control; SNP, single-nucleotide polymorphism.

mega-analysis⁹ as the discovery set and then computed in the independent Swedish subjects using the *-score* function in PLINK. We selected high-quality, relatively independent PGC SNPs with unambiguous directions of effect using a threshold of $P_T < 0.01$ (described in Ripke *et al.*⁷). For rare CNVs, we use the data from the Swedish sample and focused on the number of CNV events as burden metrics. We first evaluated the burden of known SCZ-associated CNVs that were replicated at the nominal level in the Swedish sample (1q21.1del, 3q29del, 15q13.3del, 22q11.2del, 16p11.2dup). We evaluated the burden of all >100 kb CNVs stratified by type, size and frequency categories. We fit an additive logistic regression model of disease status on RPS and rare CNV burden as predictors and genotyping batch as covariate. An additive model is justified, because the Spearman correlations between CNV burden and RPS were all near zero ($P > 0.05$) and the interaction term, when included in the logistic regression model, was not significant. To estimate the proportion of variance in case-control status accounted for by RPS and CNV burden, we computed the difference in the Nagelkerke pseudo R^2 score contrasting a full model with a reduced model in a series of logistic regression models: (1) $\text{logit}(\text{Pr}(\text{case})) \sim \text{RPS burden} + \text{CNV burden} + \text{genotyping batch}$; (2) $\text{logit}(\text{Pr}(\text{case})) \sim \text{RPS burden} + \text{genotyping batch}$; and (3) $\text{logit}(\text{Pr}(\text{case})) \sim \text{genotyping batch}$. For RPS, the pseudo R^2 contrast models (2) with (3). For CNV burden, the pseudo R^2 contrast models (1) with (2). CNV burden could also be obtained by contrasting a logistic regression model containing CNV burden plus batch covariate with (3), which gave the same results because of the independence of RPS and CNV burden.

Exploratory analyses

Bloom syndrome (OMIM #210900) is characterized by excessive homologous recombination in affected individuals and is caused by mutations in *BLM*. Using the subsample with exome sequencing genotypes,¹⁴ we compared CNV burden between individuals with and without deleterious mutations in *BLM* by fitting a linear model that had CNV burden as dependent variable, *BLM* mutation status as a predictor and genotyping batch as covariate.

Replication

We obtained replication association results from 6882 SCZ cases and 11 255 controls. Cases were from the United Kingdom CLOZUK and CardiffCOGS samples.⁸ Cases were genotyped at the Broad Institute using Illumina OmniExpress or OmniCombo arrays. Controls were from four external studies of non-psychiatric disorders. Details of the replication samples and CNV calling and QC are documented in Supplementary

Methods. Replication was attempted for all novel association regions with $P < 0.01$ in the Swedish case-control comparisons. For each region, we applied matching procedures to count the number of CNV events in the UK samples. Specifically, for single genes, we computed the counts of CNV events disrupting the gene (≥ 1 bp overlap). For all other region, we computed the counts of CNV events that overlapped the region by $>50\%$ of its length. Statistical testing for replication was by using the Fisher's exact test and the stratified Cochran-Mantel-Hänszel exact test to account for the case-control ratio difference between the Swedish and the UK samples.

RESULTS

Large chromosomal anomalies can be identified using genotyping arrays.⁶⁰ We identified four SCZ cases and three controls as genomic outliers (defined as >40 CNVs or >6 Mb, thresholds determined empirically as >3 s.d. above the sample means). Six of the seven abnormalities were confirmed using a second technology (Supplementary Table S6 and Supplementary Figure S2). One case and one control had trisomy of chr8 or chr3 (Supplementary Figure S2a and b), both of which were confirmed by using quantitative PCR.⁶⁰ Three individuals had deletion (one case) or duplication events (two controls) at 15q11.2 (20.5–22.5-Mb, Supplementary Figure S2f), which overlapped a CNV associated with mental retardation. One case (Supplementary Figure S2g) had multiple deletions that appeared to be consistent with mosaicism. Due to their large size, all seven individuals with genomic outliers were excluded from all subsequent analyses.

Extensive QC procedures (Figure 2) were used to establish a stringent CNV data set in 4719 SCZ cases and 5917 controls (Table 1, Supplementary Table S8). There was no case-control or chip bias in the proportions of CNV deletions or median CNV size. However, chip effects were observed in the mean numbers of CNVs per subject. We observed greater mean numbers of CNVs in cases than in controls with ratios that varied by chip type, most notably in the 3.9% samples that were genotyped on the older platform Affymetrix 5.0. Therefore, we controlled for chip effect in all analyses as detailed in Materials and methods section so that our findings are robust against this confounder.

Table 1. Subject and CNV characteristics

Sample characteristics	Cases	Controls
<i>Subjects (after quality control)</i>		
Sw1 (Affymetrix 5.0)	207	206
Sw2-4 (Affymetrix 6.0)	1847	2137
Sw5-6 (Illumina OmniExpress)	2665	3574
Total sample	4719	5917
Male sex	0.599	0.513
Median age at sampling	54 (45–62)	57 (48–65)
Median hospital admissions for schizophrenia	6 (3–13)	NA
Median total inpatient days	239 (79–694)	NA
Median years from first to last admission	9.7 (2.9–19.5)	NA
<i>Proportion of all CNVs that are deletions</i>		
Sw1	0.40	0.47
Sw2-4	0.37	0.36
Sw5-6	0.40	0.40
Total sample	0.38	0.38
<i>Median CNV size</i>		
Sw1	181.3	178.0
Sw2-4	182.2	188.2
Sw5-6	183.1	190.7
Total sample	182.7	188.8
<i>Mean number of CNVs per subject</i>		
Sw1	1.058	0.845
Sw2-4	1.236	1.209
Sw5-6	0.758	0.684
Total sample	0.958	0.879

Abbreviation: CNV, copy number variation. After quality control, the combined sample size is 10 636, where Sw1 = 413 (3.9%, Affymetrix 5.0), Sw2-4 = 3984 (37.5%, Affymetrix 6.0) and Sw5-6 = 6239 (58.7%, Illumina OmniExpress). Values in parentheses are interquartile ranges. Cases had significantly more males ($P < 0.0001$) and were significantly younger ($P < 0.0001$) than controls although these differences were not of large magnitude. The higher median age in controls is in the direction of greater confidence in control classification (that is, controls had greater time at risk for psychiatric hospitalization). The mean number of >500 kb CNVs and of singleton CNVs are reported in Supplementary Table S8.

Genome-wide CNV burden

We assessed the role of rare CNVs >100 kb. CNVs were stratified by type, frequency and size. We computed ORs using logistic regression with genotyping batch as a covariate. Complete results are in Supplementary Tables S9 and S10 and summarized in Figure 3. We confirmed the literature finding that SCZ cases had a significantly greater genome-wide burden of CNVs than controls: ORs of 1.07 per CNV (95% CI 1.03–1.11, $P_{emp} = 3 \times 10^{-4}$), 1.02 (95% CI 1.01–1.03, $P_{emp} = 1 \times 10^{-5}$) per gene affected by CNVs, and 1.02 (95% CI 1.01–1.03, $P_{emp} = 3 \times 10^{-5}$) per 100 kb affected by CNVs. Deletions were enriched in cases to a greater extent than duplications. The rarest CNVs (single occurrence) and the largest CNVs (>500 kb) were enriched in cases to a greater extent than other frequency or size categories. As expected, the rarest deletions (OR 1.34 per single-occurrence deletion, 95% CI 1.12–1.6, $P_{emp} = 6 \times 10^{-4}$) and the largest deletions (OR 1.4 per >500 kb deletion, 95% CI 1.16–1.7, $P_{emp} = 4 \times 10^{-4}$) showed the strongest effects albeit with broad CIs. Next, we examined the distribution of event size in the rarest deletions (Supplementary Table S11); and for all size categories (100–200 kb, 200–500 kb, >500 kb), we observed greater OR estimates using single-occurrence deletions compared with those obtained from corresponding categories using all deletions. Similarly, we examined the distribution of allele

frequency in the largest deletions (Supplementary Table S12); and for single- and 2–6-occurrence categories, we observed greater OR estimates using >500 kb deletions compared with those obtained from corresponding categories using all deletions. The highest OR was obtained in >500 kb single-occurrence deletions. These results (Supplementary Tables S11 and S12), although based on a smaller sample size, imply that size and rarity could be independent predictors of case-control status in the Swedish sample.

Finally, the largest size category (>500 kb) included CNVs in known SCZ-associated regions that were enriched in cases (Table 2). After excluding 42 CNVs in these regions, the OR was 1.17 per >500 kb CNV (95% CI 1.01–1.31, $P_{emp} = 0.033$), slightly smaller than in the full data (OR = 1.26), and was mostly due to single-occurrence deletions (OR = 1.77, 95% CI 1.11–2.82, $P_{emp} = 0.011$).

CNV loci

We examined regions known to increase risk for SCZ and other psychiatric disorders.^{10,11,13} Complete results are shown in Table 2 and are briefly summarized below. We previously verified these CNVs using exome genotyping arrays.¹⁹ We confirmed literature findings of associations between risk for SCZ and 22q11.2 deletions (OR = 16.32, 95% CI: 1.48–Inf, $P_{emp} = 0.0037$), 16p11.2 duplications (OR = 6.28, 95% CI: 1.34–59, $P_{emp} = 0.0031$) and 3q29 deletions (OR = 16.32, 95% CI: 1.48–Inf, $P_{emp} = 0.009$). Weaker evidence was observed for 15q13.3 deletions (OR = 4.39, 95% CI: 0.84–43.34, $P_{emp} = 0.05$) and 1q21.1 deletions (OR = 6.27, 95% CI: 0.7–296.4, $P_{emp} = 0.048$). We observed more deletions disrupting *NRXN1* exons in SCZ cases (two deletions in cases disrupting one exon of *NRXN1* but 0 exonic deletion in controls (Supplementary Figure S5)). We observed more duplications at 22q11.2 in controls (0 cases and 5 controls), of which three spanned the full length of the known 22q11.2 deletion locus and two spanned ~30%. The full report of 22q11.2 duplications in a careful CNV analysis of 47 005 individuals (21 138 SCZ cases and 25 867 controls) has recently been published,⁶¹ where the discovery sample included controls across studies of both psychiatric and non-psychiatric phenotypes and the replication samples were obtained from multiple sources, including our Swedish sample. Rees et al.⁶¹ reported 22q11.2 duplications as significantly less common in SCZ cases than in the general population (0.014% versus 0.085%, OR = 0.17, $P = 0.00086$) and suggested 22q11.2 duplications as the first putative protective mutation for SCZ.

Duplications at 17q12 have been implicated in autism and mental retardation, but their association with SCZ has not been previously reported. We observed more 17q12 duplications in SCZ cases (five cases and one control, OR = 6.27, 95% CI: 0.7–296.4, $P_{emp} = 0.07$). In the replication data, five cases and two controls had 17q12 duplications (OR = 4.16, 95% CI: 1.28–Inf, $P = 0.018$ in a one-sided Fisher's exact test; OR = 4.05, 95% CI: 1.35–Inf, $P = 0.024$ in a one-sided Cochran–Mantel–Hänszel exact test). Supplementary Table S13 and Supplementary Figures S3 and S4 depict the 22q11.2 and 17q12 duplications from GWAS and exome arrays, confirming these events.

We tested regions known to increase risk for developmental delay.³⁶ Of the 173 loci, 21 deletions and 16 duplications overlapped (>20%) the Swedish SCZ results (Table 2). Among the loci unique to developmental delay, no significant association with SCZ (empirical $P > 0.05$) was observed.

After excluding previously implicated loci (Table 2),^{10,11,13,35,36} we identified 15 regions nominally associated with SCZ (empirical $P < 0.01$). Five regions had substantial overlap with segmental duplications (>50%) and were excluded. None of the remaining 10 regions had strong evidence for association in the replication data set (Supplementary Table S14).

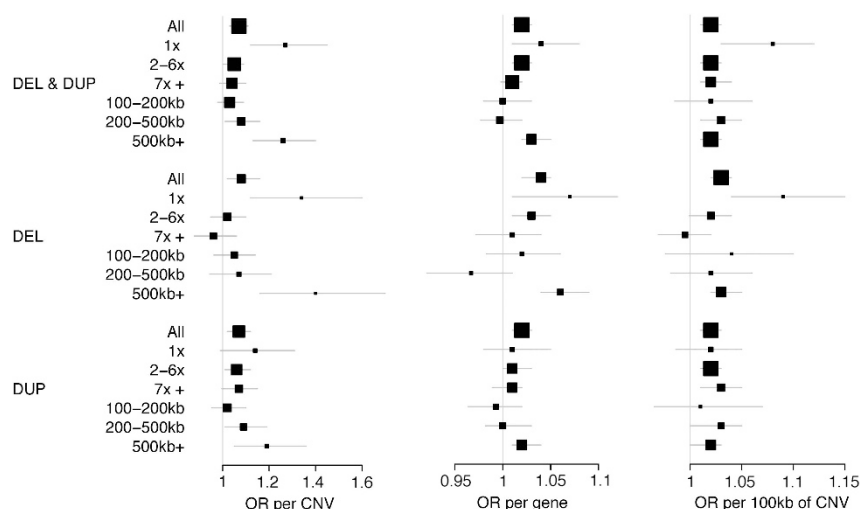


Figure 3. Genome-wide copy number variation (CNV) burden. These forest plots show odds ratio (OR) estimates and 95% confidence intervals for each burden test. The size of the square is proportional to precision. Allele frequency categories: 1 × means single-occurrence CNVs observed once in a case or control (minor allele frequency (MAF) < 0.0001). These were conservatively defined as having no overlap with any other CNVs. 2–6 × means 2–6 occurrences (MAF 0.0001–0.0005). 7 × + means ≥ 7 occurrences (MAF 0.0005–0.01).

Table 2. (a) Known pathogenic CNVs associated with psychiatric disorders (deletions)

CNV region ^a	Location (mb)	Freq _{cases}	Freq _{control}	OR (CI) ^b	EMP one-sided ^c (adjusted)	EMP two-sided ^d (adjusted)
1q21.1 ^e	chr1:145.0–148.0	5	1	6.27 (0.7–296.41)	0.048 (0.29)	
2p16.3 (<i>NRXN1</i> exons) ^e	chr2:50.1–51.2	2	0	6.27 (0.24–Inf)	0.19 (0.57)	
3q29 ^e	chr3:195.7–197.3	6	0	16.32 (1.48–Inf)	0.009 (0.022)	
7q11.23	chr7:72.7–74.1	0	0			
7q36.3 (<i>VIPR2</i>)	chr7:158.8–158.9	0	0			
15q11.2	chr15:23.6–28.4	0	0			
15q13.3 ^e	chr15:30.9–33.5	7	2	4.39 (0.84–43.34)	0.05 (0.1)	
16p13.11	chr16:15.4–16.3	3	4	0.94 (0.14–5.56)	1 (1)	1 (1)
16p11.2 distal ^e	chr16:28.2–29.0	2	1	2.51 (0.13–147.88)	0.43 (0.80)	
16p11.2	chr16:29.5–30.2	0	0			
17q12 ^e	chr17:34.8–36.2	0	0			
22q11.2 ^e	chr22:18.7–21.8	6	0	16.32 (1.48–Inf)	0.0037 (0.022)	

(b) Known pathogenic CNVs associated with psychiatric disorders (duplications)

1q21.1	chr1:145.0–148.0	2	1	2.51 (0.13–147.88)	0.36 (0.98)	
2p16.3 (<i>NRXN1</i>)	chr2:50.1–51.2	0	0			
3q29	chr3:195.7–197.3	1	0	3.76 (0.03–Inf)	0.40 (0.88)	
7q11.23	chr7:72.7–74.1	2	0	6.27 (0.24–Inf)	0.24 (0.57)	
7q36.3 (<i>VIPR2</i>) ^e	chr7:158.8–158.9	0	2	0.25 (0–6.68)	1 (1)	1 (1)
15q11.2	chr15:23.6–28.4	0	0			
15q13.3	chr15:30.9–33.5	1	2	0.63 (0.01–12.05)	1 (1)	1 (1)
6p13.11	chr16:15.4–16.3	12	6	2.51 (0.87–8.16)	0.168 (0.713)	
16p11.2 distal	chr16:28.2–29.0	2	4	0.63 (0.06–4.38)	1 (1)	0.68 (1)
16p11.2 ^e	chr16:29.5–30.2	10	2	6.28 (1.34–59)	0.0031 (0.022)	
17q12	chr17:34.8–36.2	5	1	6.27 (0.7–296.41)	0.074 (0.318)	
22q11.2	chr22:18.7–21.8	0	3	0.18 (0–3.03)	1 (1)	0.26 (0.83)

Abbreviations: CI, confidence interval; CNV, copy number variation; OR, odds ratio. ^aFor *NRXN1* in Table 2a, we considered all >100 kb deletions disrupting exons (≥1 bp overlap). For *VIPR2* and *NRXN1* in Table 2b, we considered all >100 kb CNV events that disrupted the gene (≥1 bp overlap). For all other regions, we considered all >100 kb CNV events that had >50% reciprocal overlap with a region (PLINK --cnv-union-overlap 0.5). ^bWhen 0 events occurred, ORs were estimated by applying the standard continuity correction that added a value of 0.5 to each cell of the 2 × 2 table. ^cTest was one-sided assuming higher rate in cases and statistical significance was estimated empirically by 100 000 permutations within batches. The multiple-testing-adjusted *P*-value was obtained in PLINK using the default max-T method. ^dFor simplicity, results for two-sided tests were only shown when Freq_{control} > Freq_{case}. ^eIndicates previously implicated loci in schizophrenia.

Gene set testing

Given that the rarest (single-occurrence) and the largest CNVs (>500 kb) had the strongest risks for SCZ, these subclasses were examined further. Gene set testing results

obtained from single-occurrence CNVs did not reveal additional gene sets with significant enrichment (data not shown). Gene set testing results obtained from CNVs >500 kb showed higher effect sizes compared with the corresponding

Table 3. Gene set association, previously implicated in schizophrenia

Gene set	Type	CNVs> 100 kb						CNVs> 500 Kb						
		Genes	CNVs	Genes	OR	95% CI	P _{emp}	Adj_P	CNVs	Genes	OR	95% CI	P _{emp}	Adj_P
mir137 targets (TargetScan)	DEL & DUP	1089	799	274	1.04	(0.909, 1.19)	0.3303	1	259	106	1.24	(0.969, 1.59)	0.03377	1
	DEL		228	96	1.03	(0.797, 1.32)	0.4005	1	101	48	1.43	(0.936, 2.17)	0.04573	1
	DUP		571	218	1.04	(0.888, 1.22)	0.382	1	158	72	1.13	(0.819, 1.55)	0.2149	1
Calcium signaling (KEGG:hsa04020)	DEL & DUP	178	238	45	2.48	(1.36, 4.51)	0.0004	0.041	87	19	4.39	(1.46, 13.2)	0.00042	0.047
	DEL		55	19	0.87	(0.656, 1.16)	0.856	1	26	8	1.18	(0.73, 1.91)	0.2473	1
	DUP		183	34	1.07	(0.834, 1.37)	0.2788	1	61	14	1.53	(0.992, 2.36)	0.01746	1
FMRP targets (Darnell et al. ⁴⁷)	DEL & DUP	810	913	225	1.14	(1.02, 1.27)	0.0079	0.66	286	86	1.27	(1.07, 1.51)	9.00E-04	0.095
	DEL		209	79	1.09	(0.903, 1.33)	0.1683	1	89	31	1.7	(1.08, 2.67)	0.00038	0.043
	DUP		704	185	1.16	(1.01, 1.32)	0.0162	1	197	69	1.17	(0.956, 1.43)	0.04766	13
PSD (Kirov et al. ¹²)	DEL & DUP	668	655	161	1.13	(0.982, 1.3)	0.0358	1	295	64	1.4	(1.12, 1.74)	0.00062	0.067
	DEL		174	58	1.17	(0.875, 1.57)	0.1604	1	93	26	1.57	(1, 2.45)	0.01749	1
	DUP		481	132	1.12	(0.958, 1.32)	0.0614	1	202	47	1.35	(1.05, 1.73)	0.00648	0.55
PSD/ARC (Kirov et al. ¹²)	DEL & DUP	28	103	5	1.12	(0.757, 1.66)	0.307	1	79	3	1.2	(0.759, 1.91)	0.2138	1
	DEL		47	4	1.19	(0.662, 2.12)	0.2981	1	38	3	1.1	(0.557, 2.16)	0.3793	1
	DUP		56	4	1.09	(0.644, 1.86)	0.4031	1	41	2	1.22	(0.648, 2.29)	0.2741	1
PSD/mGluR5 (Kirov et al. ¹²)	DEL & DUP	38	35	11	1.7	(0.854, 3.4)	0.0485	1	24	3	4.59	(1.56, 13.5)	0.00022	0.025
	DEL		15	6	1.77	(0.594, 5.3)	0.1635	1	10	2	16.7	(0.817, 341)	5.00E-04	0.056
	DUP		20	7	1.61	(0.658, 3.95)	0.1009	1	14	2	2.62	(0.817, 8.41)	0.02174	1
PSD/NMDAR (Kirov et al. ¹²)	DEL & DUP	61	52	22	2.37	(1.31, 4.29)	0.0010	0.11	28	11	5.8	(2, 16.8)	3.00E-05	0.0038
	DEL		19	9	2.04	(0.763, 5.46)	0.0774	1	10	5	17.27	(0.849, 351)	0.00058	0.063
	DUP		33	16	2.56	(1.22, 5.39)	0.0038	0.34	18	7	3.79	(1.24, 11.6)	0.00235	0.22
PSD/PSD-95 (Kirov et al. ¹²)	DEL & DUP	65	80	17	1.81	(1.15, 2.86)	0.0021	0.20	23	6	4	(1.34, 11.9)	0.00118	0.12
	DEL		35	9	2.87	(1.32, 6.22)	0.0011	0.11	18	5	32.18	(1.71, 604)	2.00E-05	0.0025
	DUP		45	11	1.31	(0.725, 2.35)	0.141	1	5	3	0.23	(0.026, 2.1)	0.9425	1
GWAS $P < 0.001$ (Ripke et al. ⁷)	DEL & DUP	2691	3543	819	1.04	(0.981, 1.1)	0.119	1	937	355	1.21	(1.1, 1.33)	4.00E-05	0.0049
	DEL		1210	299	1.04	(0.923, 1.16)	0.2869	1	279	110	1.31	(1.07, 1.61)	0.0041	0.3649
	DUP		2333	679	1.04	(0.977, 1.12)	0.1464	1	658	295	1.2	(1.08, 1.33)	0.00021	0.025
Mitochondrion (Kirov et al. ¹²)	DEL & DUP	193	143	52	1.14	(0.844, 1.55)	0.204	1	67	25	1.63	(1, 2.64)	0.01944	1
	DEL		60	21	2.12	(1.22, 3.67)	0.0015	0.15	40	13	4.31	(1.71, 10.9)	4.00E-05	0.0049
	DUP		83	42	0.79	(0.528, 1.17)	0.8908	1	27	17	0.61	(0.289, 1.29)	0.9201	1
MitoCarta (Pagliarini et al. ⁵⁶)	DEL & DUP	892	756	232	1.06	(0.948, 1.19)	0.1439	1	229	99	1.28	(1.04, 1.56)	0.00295	0.27
	DEL		311	86	1.18	(0.98, 1.42)	0.0277	1	105	39	1.74	(1.12, 2.71)	0.00019	0.022
	DUP		445	190	0.98	(0.835, 1.15)	0.6469	1	124	79	1.07	(0.824, 1.38)	0.3387	1

Abbreviations: ARC, activity-regulated cytoskeleton-associated complex; CI, confidence interval; CNV, copy number variation; DEL, deletions; DUP, duplications; FMRP, fragile X mental retardation protein; GWAS, genome-wide association studies; KEGG, Kyoto Encyclopedia of Genes and Genomes; NMDAR, N-methyl-D-aspartate receptor; OR, odds ratio; PSD, postsynaptic density. All tests were one-sided assuming enrichment in cases using genic CNVs. CNV = the number of events that overlapped any gene in the geneset by ≥ 1 bp. Genes = the number of unique genes in the geneset that had at least 1 genic CNV hit (≥ 1 bp overlap). OR indicates the increase in risk for schizophrenia correcting for rate and size of genic CNVs and genotyping batch effect (a continuity correction applied if necessary). P_{empir}, empirical P values were obtained in PLINK by 100 000 permutations and permuting phenotype labels within genotyping batches. Adj_P: Holm-Bonferroni multiple testing-adjusted P-values considering all 126 tests performed in Table 3 and Supplementary Table S16. The bold values indicate significant P values (P < 0.05).

Abbreviations: ARC, activity-regulated cytoskeleton-associated complex; CI, confidence interval; CNV, copy number variation; DEL, deletions; DUP, duplications; FMRP, fragile X mental retardation protein; GWAS, genome-wide association studies; KEGG, Kyoto Encyclopedia of Genes and Genomes; NMDAR, N-methyl-D-aspartate receptor; OR, odds ratio; PSD, postsynaptic density. All tests were one-sided assuming enrichment in cases using genic CNVs. CNV = the number of events that overlapped any gene in the dataset by ≥ 1 bp. Genes = the number of unique genes in the dataset that had at least 1 genic CNV hit (≥ 1 bp overlap). OR indicates the increase in risk for schizophrenia correcting for rate and size of genic CNVs and genotyping batch effect (a continuity correction applied if necessary). P_{emp} , empirical P values were obtained in PLINK by 100 000 permutations and permuting phenotype labels within genotyping batches. Adj_P: Holm–Bonferroni multiple testing-adjusted P -values considering all 126 tests performed in Table 3 and Supplementary Table S16. The bold values indicate significant P values ($P < 0.05$).

tests based on CNVs >100 kb (Table 3, Supplementary Table S16).

We hypothesized that different types of genetic variation can perturb the same biological pathways critical to the etiology of SCZ. To test this, we compared CNV rates in cases versus controls in gene sets previously implicated in SCZ (Table 3). The strongest enrichment was observed for CNVs (deletions and duplications combined) in neuronal calcium channel signaling (OR = 2.48, 95% CI: 1.36–4.51, $\text{adj_}P_{\text{emp}} = 0.041$ for events >100 kb; OR = 4.39, 95% CI: 1.46–13.2, $\text{adj_}P_{\text{emp}} = 0.047$ for events >500 kb). For CNVs >500 kb in SCZ cases, 56 events overlapped 11 genes of the calcium signaling pathway, among which 17 (30%) events overlapped (by >50%) known risk CNV loci (Supplementary Table S17). A significant enrichment in FMRP targets⁴⁴ (OR = 1.7, 95% CI: 1.08–2.67, $\text{adj_}P_{\text{emp}} = 0.043$) was observed for deletions >500 kb, of which 76 case deletions (36 in known loci) overlapped 28 genes (Supplementary Table S17). Previously, the calcium channel and FMRP targets were enriched for rare exonic mutations in exome sequencing of half of the Swedish samples^{7,14,47} and were enriched for common variants in a meta-analysis of the Swedish samples and independent PGC samples (a total of 13 833 cases with SCZ and 18 310 controls).^{7,14,47} We then examined the overlap between genes affected by common variants and by rare CNVs that are driving the enrichment signals in these two shared pathways. Supplementary Table S15 shows that common variants affected more genes than rare CNVs did and that common variants and rare CNVs had no overlap for calcium channel and a small overlap for FMRP targets. These results suggest that different types of risk alleles were independently enriched in SCZ cases than in controls.

For genes found at the PSD, we observed increased burden for the gene set used by Kirov *et al.*¹² (OR = 1.4, 95% CI: 1.12–1.74, $\text{adj_}P_{\text{emp}} = 0.067$ for >500 kb deletions and duplications combined) and a stronger enrichment using the GO category 'postsynaptic density' (OR = 6.74, 95% CI: 1.66–27.3 for >500 kb deletions, Table 4). We next tested gene sets comprising components of the PSD as in Kirov *et al.*¹² and observed significant enrichment ($\text{adj_}P_{\text{emp}} < 0.05$) in the *N*-methyl-D-aspartate receptor gene set and in genes encoding mGluR5 for >500 kb deletions and duplications combined, and in the PSD-95 (postsynaptic density protein 95) complex for >500 kb deletions only (Table 3). Most of the case CNVs (~73%) overlapped three known risk CNV loci (3q29, 16p11.2, 22q11.2) and overlapped genes previously enriched for case *de novo* CNVs¹² (Supplementary Table S17). Overlap between *de novo* CNVs with 3q29 and 16p11.2 was previously reported.¹²

Gene products often localize to particular subcellular compartments. Identifying compartments enriched for genes impacted by CNVs in SCZ patients could provide greater insight into pathophysiological mechanisms related to the disorder. Besides the PSD genes, we tested additional gene sets enriched for *de novo* CNVs in Kirov *et al.*¹² (Supplementary Table S16) and observed significant enrichment for gene products localized to cytoplasm (OR = 3.38, 95% CI: 1.49–7.68, $\text{adj_}P_{\text{emp}} = 0.012$ for duplications >500 kb) and mitochondria (OR = 4.31, 95% CI: 1.17–10.9, $\text{adj_}P_{\text{emp}} = 0.0049$ for deletions >500 kb). Given the potential role of mitochondrial dysfunction in psychiatric disorders,^{62,63} we further tested human nuclear-encoded mitochondrial genes in MitoCarta and found significant enrichment of case deletions (1.74, 95% CI: 1.12–2.71, $\text{adj_}P_{\text{emp}} = 0.022$ for deletions >500 kb, Table 3). Half of the case CNVs (~51%) overlapped known CNV risk loci (Supplementary Table S17).

As many CNVs associated with SCZ also show pleiotropic effects for autism and mental retardation, we tested the genes implicated in these disorders in the Swedish sample (Supplementary Table S16). Large CNVs (>500 kb duplications and deletions combined) in SCZ cases were enriched in genes implicated in mental retardation (OR = 2.71, 95% CI: 1.41–3.34, $\text{adj_}P_{\text{emp}} = 0.0049$).

Approximately 33% case CNVs overlapped known CNV risk loci (Supplementary Table S17), which is not surprising as many of the MR genes have been identified by genomic re-arrangements.

SCZ is often associated with cognitive deficits. Therefore, we tested whether CNVs in SCZ patients were enriched for genes associated with cognitive ability in humans and neurological or behavioral phenotypes in mice (Supplementary Table S16). We observed significant enrichment of large deletions (>500 kb) in synaptic genes based on functional group analysis⁴⁸ (OR = 1.67, 95% CI: 1.15–2.43, $\text{adj_}P_{\text{emp}} = 0.036$). Although not significant based on multiple-testing adjustment, increased burden of deletions was observed in genes for which knockout in mouse yields a neurological or behavioral phenotype (OR = 1.26, 95% CI: 1.06–1.49, $P_{\text{emp}} = 0.0021$ for deletions >100 kb; OR = 1.53, 95% CI: 1.11–2.1, $P_{\text{emp}} = 0.00099$ for deletions >500 kb).

The markedly increased sample size of this study relative to others provides the opportunity to identify novel biological pathways associated with SCZ. Therefore, we performed a series of exploratory analyses using gene sets based on KEGG, GO and TargetScan (targets of microRNAs other than miR-137). Table 4 lists the top ranking gene sets that were significantly enriched in cases than in controls (all had $P < 0.0002$). For KEGG and TargetScan, all had q -value < 0.01, implying that < 1% of significant tests or < 0.27 test will result in false discoveries; and for GO, all had q -value < 0.004, implying that < 0.4% of significant tests or < 0.59 test will result in false discoveries. These gene sets included multiple elements of the immune system (T-cell receptor signaling, hematopoietic cell lineage, positive regulation of monocyte proliferation), members of chromatin remodeling complexes (for example, *HDAC9*, *NCOR1*) and a novel association with target genes of microRNA miR-10a as well as basic elements of microRNA processing machinery necessary for gene silencing (that is, *DGCR8*, *ERI1*, *NCBP2*). We investigated the potential role of miR-10a using the Swedish GWAS data.⁷ However, unlike miR-137, no significant GWAS hit was found in the vicinity of miR-10a, and the targets of miR-10a were not enriched for smaller GWAS P -values (data not shown).

Rare CNVs and significant GWAS loci

We asked whether CNVs were enriched in cases compared with controls in the genes implicated by GWAS meta-analysis of the Swedish samples and independent PGC samples (a total of 13 833 cases with SCZ and 18 310 controls).⁷ For 2619 genes with GWAS $P < 10^{-3}$, we observed significant enrichment for large CNVs (OR = 1.21, 95% CI: 1.1–1.33, $\text{adj_}P_{\text{emp}} = 0.0049$ for >500 kb deletions and duplications combined, Table 3).

Rare CNV and common variant burden

We computed the Spearman correlations between rare CNV burden and common variant burden (that is, RPS), which were all near zero and not statistically significant ($P > 0.05$). Thus, the two types of genetic burden are uncorrelated. The relative impact of rare CNV burden and RPS burden was quantified by fitting multiple logistic regression models and estimating the effect size (R^2). The two classes of variation were significantly, independently and additively enriched in SCZ cases compared with controls (Supplementary Table S18). Figure 4 and Supplementary Table S19 show the R^2 values using CNV burden at known SCZ-associated loci (1q21.1del, 3q29del, 15q13.3del, 22q11.2del, 16p11.2dup and all five loci combined). Supplementary Figure S6 and Supplementary Table S20 show the R^2 values using burden of >100 kb single-occurrence deletions, >500 kb deletions and CNVs in other categories. In summary, RPS accounted for at least an order of magnitude more variance than rare CNVs of strongest effects in this sample. Similar results were obtained using gene count and total length as CNV burden metrics (data not shown).

Table 4. Top-ranking significant gene sets from the discovery analysis

Source	Gene set		Type	CNVs > 100 kb					CNVs > 500 kb				
	Name	Genes		No. of CNVs	OR	95% CI	P _{emp}	Genes within CNVs (genes)	CNVs	OR	95% CI	P _{emp}	Genes within CNVs (genes)
KEGG	hsa04660 T-cell receptor signaling pathway	108	DEL	29	4.42	(1.61, 12.2)	1e-04	CBLB,DLG1,LAT,MAP3K14,MAP3K7,MAPK3,NFAT5,NRAS,PAK2,PDCD1,PPP3CA,RELA,VAV3 (13)	19	15.72	(0.87, 285)	4e-05	CBLB,DLG1,LAT,MAP3K7,NFAT5,PAK2,PPP3CA (7)
KEGG	hsa04640 Hematopoietic cell lineage	85	DEL	—	—	—	—	—	17	14.4	(1.9, 109)	2e-05	CD19,CD36,GP1BB,IL7R,TFRC (5)
KEGG	hsa04621 NOD-like receptor signaling pathway	58	DUP	22	9.27	(2.17, 39.7)	1e-05	CCL5,CXCL1,CXCL2,ERBB2,IPHS90AA1,IL8,MAPK3,MAPK8,NAIP,NLRC4,RIPK2 (11)	15	7.05	(1.6, 31)	2e-04	CXCL1,CXCL2,IL8,MAPK3,MAPK8,NAIP,RIPK2 (7)
TargetScan	hsa-miR-10a	144	DEL& DUP	—	—	—	—	—	50	2.86	(1.54, 5.32)	3e-05	AP002478.1,BCL2L1,BCR,C10orf68,C16orf54,CEP350,CLEC18A,CNST,COL4A4,C5MD1,ELOVL2,EPHA2,GJA5,LCA5,PVRL3,SDPR,SLC04C1,TFRC,TIAM1,ZNF254 (20)
GO	GO:16458 gene silencing	71	DEL	14	31.52	(1.67, 595)	1e-05	DGCR8,ERI1,NCBP2 (3)	14	28.21	(1.48, 537)	1e-05	DGCR8,ERI1,NCBP2 (3)
GO	GO:200602 regulation of interphase of mitotic cell cycle	162	DEL	17	17.34	(2.31, 130)	1e-05	ATM,CDC45,DLG1,PRMT2,RPL24,USP17L2 (6)	13	26.17	(1.36, 504)	1e-05	CDC45,DLG1,RPL24 (3)
GO	GO:42471 ear morphogenesis	99	DEL	12	28.67	(1.5, 548)	5e-05	CDH23,GBX2,PTPRQ,SOBP,TBX1 (5)	—	—	—	—	—
GO	GO:14069 postsynaptic density	100	DEL	—	—	—	—	—	25	6.74	(1.66, 27.3)	1e-05	DLG1,DLGAP1,FBXO45,LIN7A,MAGI2,P2RX6,SEMA4C (7)
GO	GO:16327 apicolateral plasma membrane	109	DEL	—	—	—	—	—	24	9.61	(2.23, 41.4)	1e-05	CLDN23,CLDN5,DLG1,FRMPD2,JAM2,LIN7A,MAGI2,MICALL2,PMP22,PVRL3 (10)
GO	GO:287 magnesium ion binding	179	DEL	—	—	—	—	—	24	6.71	(1.66, 27.1)	2e-05	ABL2,COMT,FANT1,MAP3K7,NT5C3,STK38L,TSSK2 (7)
GO	GO:32526 response to retinoic acid	101	DEL	—	—	—	—	—	14	28.67	(1.51, 546)	1e-05	ABL2,PDGFA,TBX1,TFRC (4)
GO	GO:10498 proteasomal protein catabolic process	190	DUP	—	—	—	—	—	23	8.39	(2.51, 28.1)	2e-05	ANAPC1,CRBN,DERL3,FBXO45,KCTD13,MAD2L1,PSMA7,STUB1,UBB,WWP2 (10)
GO	GO:16052 carbohydrate catabolic process	131	DUP	—	—	—	—	—	14	6.88	(2.04, 23.3)	2e-05	AGL,ALDOA,ALDOB,HK2,OGDHL,PGM1 (6)
GO	GO:16585 chromatin remodeling complex	114	DUP	—	—	—	—	—	22	26.31	(3.58, 194)	1e-05	BAZ1B,DPIY30,HDAC9,INO80B,INO80E,NCOR1,SMARCA2,SMARCB1,USP22 (9)
GO	GO:2755 MYD88-dependent Toll-like receptor signaling pathway	75	DUP	—	—	—	—	—	14	17.55	(2.35, 131)	1e-05	MAP2K3,MAPK3,MAPK8,RIPK2,UBB (5)

Abbreviations: CI, confidence interval; CNV, copy number variation; DEL, deletions; DUP, duplications; GO, gene ontology; OR, odds ratio. The number of genesets (N) tested within each database: KEGG: N = 228, TargetScan: N = 87, and GO: N = 1499. All the gene sets listed here were significantly enriched in cases than in controls based on P-values. For KEGG and TargetScan, all have q-value < 0.01, implying that < 1% of significant tests or < 0.27 test will result in false discoveries; and for GO, all have q-value < 0.004, implying that < 0.4% of significant tests or < 0.59 test will result in false discoveries. All tests were one-sided assuming enrichment in cases using genic CNVs. No. of CNVs = the number of events that overlapped any gene in the geneset by ≥ 1 bp. No. of genes = the number of unique genes in the geneset that had at least 1 genic CNV hit (≥ 1 bp overlap). OR indicates the increase in risk for SCZ correcting for rate and size of genic CNVs and genotyping batch effect (a continuity correction applied if necessary). P_{emp}, empirical P-values were obtained in PLINK by 100 000 permutations and permuting phenotype labels within genotyping batches.

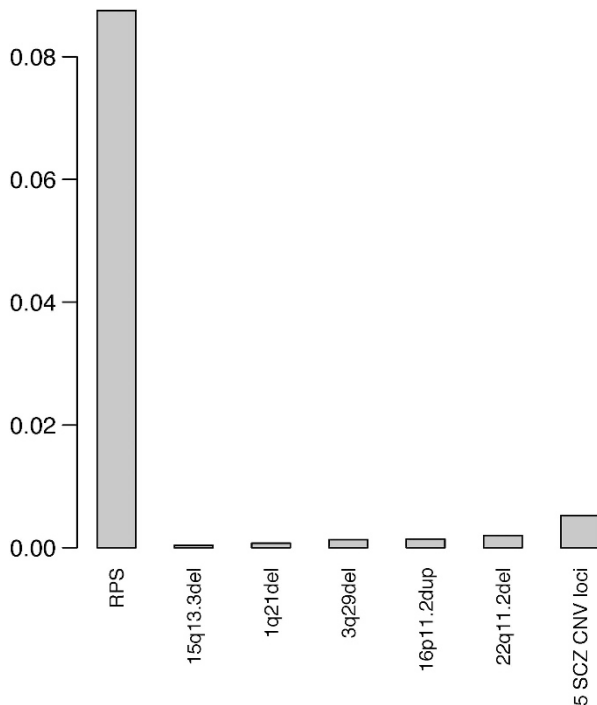


Figure 4. Relative impact of rare copy number variation (CNV) burden and common variant allelic burden. We computed the difference in the Nagelkerke pseudo R^2 score to estimate the proportion of variance of case-control status in the Swedish samples accounted for by the common variant allelic burden (risk profile scores (RPS)) and by the rare CNV burden (as measured by the number of CNV at known schizophrenia (SCZ)-associated loci). We examined CNV burden of 1q21.1del, 3q29del, 15p13.3del, 22q11.2del, 16p11.2dup, individually and combined. The y axis of the barplot shows the estimates of effect size (that is, Nagelkerke pseudo R^2). RPS accounted for at least an order of magnitude more variance than rare CNVs in this sample.

Exploratory analyses

We investigated the impact of deleterious mutations in *BLM*. Bloom syndrome is a rare Mendelian disorder characterized by genomic instability and excessive homologous recombination in affected individuals and is caused by recessive mutations in *BLM*. From exome sequencing in a subset of this sample (2222 SCZ cases and 2278 controls post-QC), there were 30 cases and 33 controls with a disruptive exonic mutation in *BLM*.¹⁴ The presence of a *BLM* mutation was not associated with case-control status ($P=0.88$ chi-squared test), but individuals with *BLM* mutations had greater CNV burden compared with individuals without mutations in *BLM* (Supplementary Table S21). For genic deletions, we observed a significantly ($P=0.0019$) greater burden (as measured by the number of impacted genes) in individuals with deleterious *BLM* mutations (with the presence of a deleterious *BLM* mutation increasing CNV genic deletion burden by a mean of 0.96).

DISCUSSION

This sample from Sweden provided an ideal CNV screening set as it is one of the largest SCZ samples yet collected in a single country and is relatively homogenous and genomically well characterized. Furthermore, we had the opportunity to validate CNVs using exome array data from the same samples as well as test for replication using large scale samples from the United Kingdom.⁶¹

Consistent with previous reports, our results confirm increases in CNV burden in cases and recapitulate known specific CNV risk loci. We also identified an association of duplications at 17q12 with SCZ that is reciprocal of the known 17q12 deletion. We did not identify any novel CNV loci as enriched among SCZ cases. Our power analyses suggest that most rare and recurrent CNVs carrying moderate-to-high risk have been discovered with GWAS arrays (Figure 1). The CNV burden analyses imply that additional recurrent CNVs do exist; as these were among the rarest events, far larger sample sizes will be required for their confident identification.

Our primary analyses applied a stringent size filter (≥ 100 kb and spanning ≥ 15 probes), because these events can be most reliably detected from GWAS arrays.^{11,13} For secondary CNV analyses, we used more liberal thresholds (≥ 20 kb and spanning ≥ 10 probes) because of tradeoffs between sensitivity and specificity. However, we did not observe additional novel findings that exceeded genome-wide significance.

Gene set enrichment testing replicated a number of findings from the literature and identified novel biological processes potentially related to SCZ pathophysiology. Consistent with previous GWAS, exome sequencing and CNV findings, we found enrichment for neuronal calcium channel signaling, FMRP-binding partners, glutamate receptors (*N*-methyl-D-aspartate and mGluR5). The FMRP targets were also linked to synaptic function and autism.⁴⁴ Novel pathways identified included: signaling components within the immune system not encoded by the major histocompatibility complex region previously associated with SCZ (T-cell receptor signaling, hematopoietic cell lineage, positive regulation of monocyte proliferation), new microRNA pathway (miR-10a) along with basic microRNA-processing elements (for example, *DGCR8*, *ERI1* and *NCBP2*), and members of chromatin remodeling complexes (for example, *HDAC9*, *NCOR1*). These novel pathways underscore the importance of the immune system and microRNA-based regulation of gene expression for SCZ etiology and suggest that major histocompatibility complex and miR-137 may be just the first components of larger biological networks yet to be identified. Furthermore, the detection of multiple pathways involved in transcriptional regulation is consistent with the SCZ GWAS results, where the vast majority of associated loci are thought to tag regulatory variation.

On the phenotype level, we found that CNVs in SCZ patients are enriched for genes associated with mental retardation, cognitive ability in humans as well as neurological or behavioral phenotypes in knockout mice. These results are consistent with the pleiotropic effects of large, rare CNVs previously described in the human genetics literature. Furthermore, the mouse finding suggests that murine models provide a biologically relevant system to examine the pathological effects of gene dosage (via knockout or overexpression) on brain and behavior. On the subcellular compartment level, we identified enrichment for gene products localized to three regions: the PSD, mitochondria, and cytoplasm. The synaptic enrichments are consistent with pathways mentioned above (neuronal calcium, glutamate and FMRP signaling) as well as a growing body of literature implicating genes related to synaptic transmission in SCZ and bipolar disorder.^{7,64,65}

Mitochondria generate ATP by oxidative phosphorylation and have important roles in the regulation of cellular calcium levels, steroid synthesis, production of free radicals and regulation of apoptosis. Brain is highly dependent on ATP generated by mitochondria. Mitochondrial dysfunction and a disturbance of energy metabolism have been observed in SCZ patients and, likewise, mitochondrial disorders can present with psychotic, affective and cognitive symptoms.^{62,63} Our data reveal that nuclear-encoded genes that localize to the mitochondria are over-represented among large, rare CNVs in SCZ patients, lending credence to the mitochondrial dysfunction hypothesis.

By exome sequencing of >5000 of these subjects, Purcell *et al.*¹⁴ found that three classes of variation, as measured by rare CNV burden, common variant/RPS burden and rare exonic variant burden, were uncorrelated and independently and additively enriched in SCZ cases compared with controls. Consistent with this report,¹⁴ we found that rare CNV burden and common variant/RPS burden are independently and additively enriched in SCZ cases. We note that it is currently unknown how environmental factors^{5,6,66} interact with genetic burden. Consistent with Purcell *et al.*,¹⁴ RPS burden accounted for at least an order of magnitude more variance than rare CNVs in this sample. This suggests that while the SCZ-associated rare and large CNVs are much more penetrant individually than SCZ-associated common SNPs, they contribute much less to the overall population risk of SCZ than do the SNPs.

In the exome sequencing study, Purcell *et al.*¹⁴ found increased burden of rare exonic mutations in genes implicated by GWAS and in a known CNV risk locus 3q29/DLG1. Here we found increased burden of rare CNVs in genes implicated by GWAS. GWAS results are robust, as they were based on a meta-analysis of the Swedish samples and a much larger independent PGC samples. The associated common variants were far more distributed both in more subjects and from more sequences in the genome than rare exonic variants or large rare CNVs. The burden of rare exonic variants, common variants and rare CNVs were uncorrelated and independent. Together, these findings suggest that multiple lines of genomic inquiry—genome-wide screens for CNVs, common variation and exonic variation—are converging on similar sets of pathways and/or genes. This convergence helps to increase confidence in the robustness of the findings and provides results relevant to resolving the rare ‘versus’ common variation debate. Further work will be required to characterize the potential mechanisms of these pathways.

We observed increased genic deletions in individuals with exonic *BLM* mutation, which is consistent with the fact that *BLM* mutations increase homologous recombination in the affected individuals. To identify genomic loci influencing individual differences in CNV burden, future investigation using large-scale trio data would be informative in order to assess how parental genotypes associate with meiotic CNVs in offspring.

We believe it is possible that CNV discovery for SCZ has entered a difficult phase where almost all low-hanging fruit have been collected (that is, large, recurrent CNVs measurable by SNP arrays). Increasing CNV discovery could well await dramatic improvements in sample size, technology and cost. The detection of large, very rare recurrent CNVs will require marked increases in sample sizes. The application of whole-genome sequencing to large case-control samples remains expensive but offers the possibility of confident identification of CNVs as small as 1 kb, particularly those that impact exons in single genes.⁶⁷ CNV technologies based on genotyping arrays do not perform well for copy number polymorphisms (frequency >0.01),⁶⁸ and it is certainly possible that this class of variation has explanatory power in SCZ but is currently not well measured.

CONFLICT OF INTEREST

PFS was on the SAB of Expression Analysis (Durham, NC, USA). PS is on the Board of Directors of Catalytic, Inc. The other authors declare no conflict of interest.

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